IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Klimpel et al. Application No.: 09/853,530 Filed: May 9, 2001 For: TARGETING ANTIGENS TO THE MHC CLASS I PROCESSING PATHWAY WITH AN ANTHRAX TOXIN FUSION PROTEIN	Examiner: Schwadron Technology Center/Art Unit: 1644 Declaration of Stephen Leppla under 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Stephen H. Leppla, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true.

2. I hold a Ph.D. (1969) from the University of Wisconsin, and a Bachelor of Science (1963) from the California Institute of Technology. I am presently Chief, Bacterial Toxins and Therapeutics Section, at the National Institute of Allergy and Infectious Diseases ("NIAID") of the National Institutes of Health ("NIH"). My field of expertise is Bacterial Toxins and Therapeutics. I have authored over one hundred and thirty publications in the field of bacterial toxins, and am a member of the NIAID Working Group on Anthrax Vaccines; the Working Group on Recombinant Toxins, NIH Office of Biotechnology Activities, OD, NIH; the American Society for Microbiology;

and the American Academy of Microbiology. A true copy of my *Curriculum Vitae* is attached hereto as Exhibit A.

3. The present invention relates to the surprising discovery that the anthrax toxin system can be used to deliver full length viral proteins as exogenous antigens to the cell cytosol for processing and presentation by MHC class I molecules to cytotoxic T lymphocyte cells (CTLs) to elicit a cytotoxic T lymphocyte immune response specific for the viral protein. In particular, the invention provides the first evidence that an anthrax toxin fusion protein comprising a full length viral protein-anthrax lethal factor (LF) fusion bound to anthrax protective antigen (PA) is translocated into the cell, and that ***the full length viral protein is efficiently processed*** into multiple epitopes by the MHC class I processing pathway and the epitopes of the full length viral antigen are presented by MHC class I molecules to CTLs. The use of full length viral protein has the advantage of providing multiple epitopes that are recognized by more than one MHC class I allele.

4. I am a named inventor on the above-referenced patent application. I have read and am familiar with the contents of the subject patent application. I have also read the Office Action received from the United States Patent and Trademark Office dated December 7, 2004. It is my understanding that the Examiner is concerned that the claimed anthrax toxin fusion proteins are obvious over WO 94/18332 ("Leppla *et al.*") in view of WO 95/03414 ("Noteborn *et al.*"). Specifically, the Examiner alleges that Leppla *et al.* discloses all elements of the claimed anthrax toxin fusion proteins except a viral protein and further alleges that Noteborn *et al.* discloses an immunoconjugate containing a viral toxin.

5. This declaration is provided to clarify for the Examiner the distinction between an MHC class I immune response and an MHC class II immune response and to demonstrate that Leppla *et al.* and Noteborn *et al.* alone or in

combination do not disclose that an anthrax toxin can be used to deliver a full-length viral protein to a cytosolic MHC class I processing pathway for induction of a CTL immune response, that one of skill in the art would have no motivation to combine the disclosures of Leppla *et al.* and Noteborn *et al.* to practice the presently claimed invention, and that one of skill in the art would not have had a reasonable expectation of success of generating the presently claimed compositions by combining the disclosures of Leppla *et al.* and Noteborn *et al.*

6. It is well known in the art that immune responses are based on two distinct pathways: MHC class I and MHC class II (*see*, Abbas *et al.*, CELLULAR AND MOLECULAR IMMUNOLOGY 118-119 and 133 (Martin Wonsiewicz ed., W. B. Saunders 1991), copy enclosed as Exhibit B). "[E]ndogenously synthesized antigens end up associated with class I MHC and exogenously synthesized and endocytosed antigens end up associated with class II MHC." *Id.* at 133. ***CTL*** responses specific for a particular antigen are produced following presentation by MHC ***class I*** molecules to CTLs. *Id.* at 119. In contrast, antibodies specific for a particular antigen are produced following presentation of epitopes by MHC class II molecules to helper T cells. *Id.* at 118.

7. The present application provides the first evidence that a bacterial toxin system (anthrax toxin) can be used to ***exogenously*** introduce a full length viral protein into the cytosol for processing via the MHC class I pathway and presentation by MHC class I molecules to CTLs. The processing of the exogenously introduced full length viral protein and presentation to CTLs via the MHC class I pathway, was demonstrated by treating antigen-presenting cells with lactacystin, which inhibits proteasome function required for cytosolic MHC class I processing (*see,* specification at page 3, lines 7-11). The present inventors are therefore the first to show that a full length viral protein, fused to LF and translocated into a cell by anthrax toxin, is ***processed*** by the cytosolic MHC class I pathway and presented by MHC class I molecules to CTLs.

8. Leppla *et al.* teaches methods for cellular delivery of small fragments of proteins or peptide epitopes, rather than full viral length proteins, using anthrax toxins comprising PA and a LF-viral protein fusion proteins. Anthrax toxin is a binary bacterial toxin comprising two proteins: LF and PA. PA binds to the cellular receptor and is cleaved, revealing an LF binding site. LF binds to PA, forming anthrax toxin which is translocated into the cell. At page 3, ¶7, lines 9-10 of the Office Action, the Examiner asserts that processed PA is created when the anthrax PA is administered *in vivo* and appears to equate cleavage of PA with processing an antigen into epitopes. However, cleavage of PA reveals its LF binding site and does ***not*** generate epitopes of PA for presentation by MHC class I molecules to CTLs. Leppla *et al.* teaches generic compositions where peptides or protein fragments are fused to LF for cellular translocation, via PA binding for delivery of an activity to a cell, *e.g.*, a cytotoxic activity. Leppla *et al.* does not teach the use of anthrax toxin fusion proteins for eliciting a CTL response specific for a viral protein by delivering full length viral proteins to the cytosolic MHC class I pathway for processing of the viral protein into epitopes and presentation of the epitopes by MHC class I molecules to CTLs.

9. Noteborn *et al.* teaches methods for delivery of a crude cellular extract containing a chicken anemia viral ("CAV") protein to induce an antibody response specific for the CAV protein (*see*, page 13, line 14 to page line 24; page 19, line 14 to page 21, line 34; and page 24, line 11 to page 25, lines 7). Noteborn *et al.* does not disclose delivery of any fusion protein to induce an immune response. Moreover, as explained in ¶6 above, an antibody response is produced via the MHC ***class II*** pathway of a cell. Noteborn *et al.* therefore, does not disclose delivery of an exogenous viral antigen to a cell for processing via the MHC class I pathway and presentation by MHC class I molecules to CTLs. Noteborn *et al.* also teaches delivery of a cytotoxic CAV protein to a cell to deliver the cytotoxic effect of the ***native*** protein (*i.e.*, apoptotic effect) to the cell (*see*, page 9, line 17-18; page 25, line 9 to page 26, line 29 and page 28, line 6 to page 30, line 8). In contrast to the presently claimed anthrax toxin fusion proteins, the

CAV protein of Noteborn *et al.* is not processed by the cytosolic MHC class I pathway and presented by MHC class I molecules to CTLs, *i.e.*, the apoptosis disclosed in Noteborn *et al.* is ***not*** a CTL response specific for the CAV protein. Therefore, Noteborn *et al.* also does not teach the use of anthrax toxin fusion proteins for eliciting a CTL response specific for a viral protein by delivering full length viral proteins to the cytosolic MHC class I pathway for processing of the viral protein into epitopes and presentation of the epitopes by MHC class I molecules to CTLs.

10. One of skill in the art would have no motivation to combine the disclosure of Leppla *et al.* with the disclosure of Noteborn *et al.* to make the presently claimed compositions. As explained in ¶ 7 above, prior to the disclosure of the instant application, one of skill in the art would not have expected that an exogenously introduced full length protein could be processed and presented via the cytosolic MHC class I pathway. Indeed, Noteborn *et al.* confirms the state of the art by disclosing that a MHC class II-mediated antibody response is induced following exogenous delivery of a CAV protein to a cell. Therefore, one of skill in the art would have no motivation to combine the disclosures of Leppla *et al.* and Noteborn *et al.* to make the presently claimed anthrax toxin fusion proteins for exogenously delivering full length viral proteins to the cytosolic MHC ***class I*** pathway for processing of the viral protein into epitopes and presentation of the epitopes by MHC class I molecules to CTLs.

11. Even if one of skill in the art were to combine the disclosures of Leppla *et al.*, and Noteborn *et al.* there would be no reasonable expectation of success in generating the presently claimed compositions. Delivery of a full length viral protein to a target cell using the presently claimed compositions would lead to processing and presentation of the viral protein by the target cell and subsequent generation of a MHC class I-mediated cytotoxic T cell response specific for the viral protein, but would neither kill the target cell as disclosed in Leppla *et al.* and Noteborn *et al.* nor generate a class II mediated antibody response against viral protein as disclosed in Noteborn *et al.* Thus,

modification of the compositions disclosed by Leppla *et al.* and Noteborn *et al.* to deliver viral proteins to a target cell would render the Leppla *et al.* and Noteborn *et al.* compositions unsuitable for their intended purposes.

12. In conclusion, the present application provides the first evidence that full length viral proteins fused to anthrax toxin are translocated into the cell, enter the cytosolic MHC ***class I*** processing pathway, and are subsequently presented by MHC class molecules to CTLs. The disclosures of Leppla *et al.* and Noteborn *et al.* alone or in combination do not disclose translocation of an exogenous antigen into a cell for processing and presentation via the MHC class I pathway. Thus, it is my opinion that the teachings of Leppla *et al.* in view of Noteborn *et al.* do ***not*** provide one of skill in the art with motivation to practice the claimed invention, nor a reasonable expectation of success for one of skill in the art in practicing the claimed invention.

13. The Declarant has nothing further to say.

Dated: 1 June 05 By: _____
Stephen H. Leppla, Ph.D.

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CURRICULUM VITAE

Stephen H. Leppla, Ph.D.

EDUCATION

1959-63	B.S. Biology, California Institute of Technology
1963-69	Ph.D. Biochemistry, Biochemistry Department University of Wisconsin, Madison Characterization of yeast ribonucleic acids

EMPLOYMENT HISTORY

1969-71	National Institutes of Health Postdoctoral Fellow Dept. of Molecular Biology, University of California, Berkeley, CA. Studies of the control of RNA synthesis in bacteria, using chemical and genetic techniques. Terminal nucleotide analysis of viral RNAs.
1971-73	Research Associate, Division of Biological and Medical Sciences, Brown University, Providence, RI. Biochemistry of schistosomiasis. Isolation of enzymatically and serologically-active materials.
1974-89	Research Chemist (and Division Chief during 1983-1985) Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD. Characterization of bacterial toxins.
1989-2001	Research Chemist, Oral Infection and Immunity Branch National Institute of Dental and Craniofacial Research National Institutes of Health, Bethesda, MD. Structure and function of bacterial protein toxins.
2001-present	Senior Investigator Chief, Bacterial Toxins and Therapeutics Section National Institute of Allergy and Infectious Diseases National Institutes of Health, Bethesda, MD. Structure and function of bacterial protein toxins.

MEMBERSHIPS IN PROFESSIONAL SOCIETIES

1974-present	American Society for Microbiology
1985-present	American Academy of Microbiology

OTHER ACTIVITIES

1980-1987	Member, Editorial Board, Infection and Immunity
1987-1991	Editor, Infection and Immunity
1995-1998	Member, NIH Institutional Biosafety Committee
1998-	Member, NIAID Working Group on Anthrax Vaccines
2000-	Member, Working Group on Recombinant Toxins, NIH Office of Biotechnology Activities, OD, NIH

Publications:

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3. Lewandowski, L. J. and **S. H. Leppla**.
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14. Middlebrook, J. L., R. B. Dorland, **S. H. Leppla**, and J. D. White.
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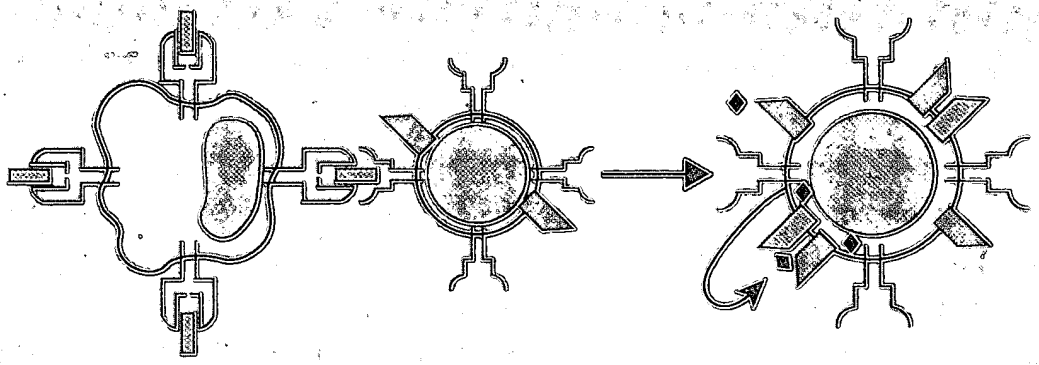

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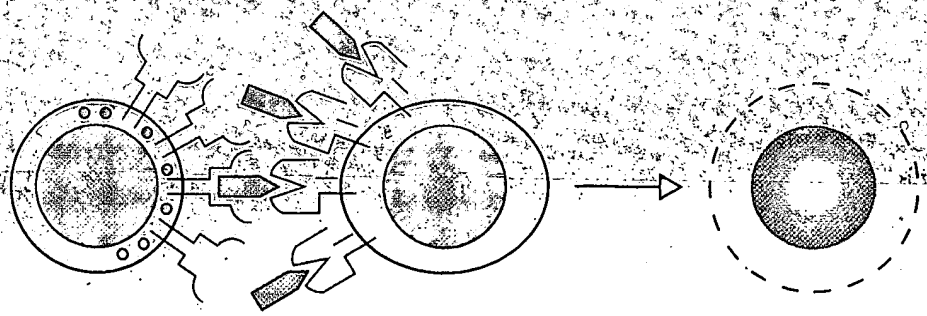

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periments established MHC restriction of antigen recognition by helper T cells and CTLs:

1. T cells from an antigen-primed guinea pig of one inbred strain proliferate in response to antigen *in vitro* only if macrophages from the same strain are present. These proliferating T cells are mostly helper cells. Subsequent analyses using inbred and congenic strains of mice revealed that in order to present antigens to helper T cells, the APCs have to express class II MHC molecules that are seen as self by the T cells (Fig. 6-1). Such experiments, and others using purified and monoclonal T cell populations from mice and humans, have established that *antigen recognition by helper T cells is class II MHC-restricted.*

2. *In vivo* experiments with inbred mice utilizing

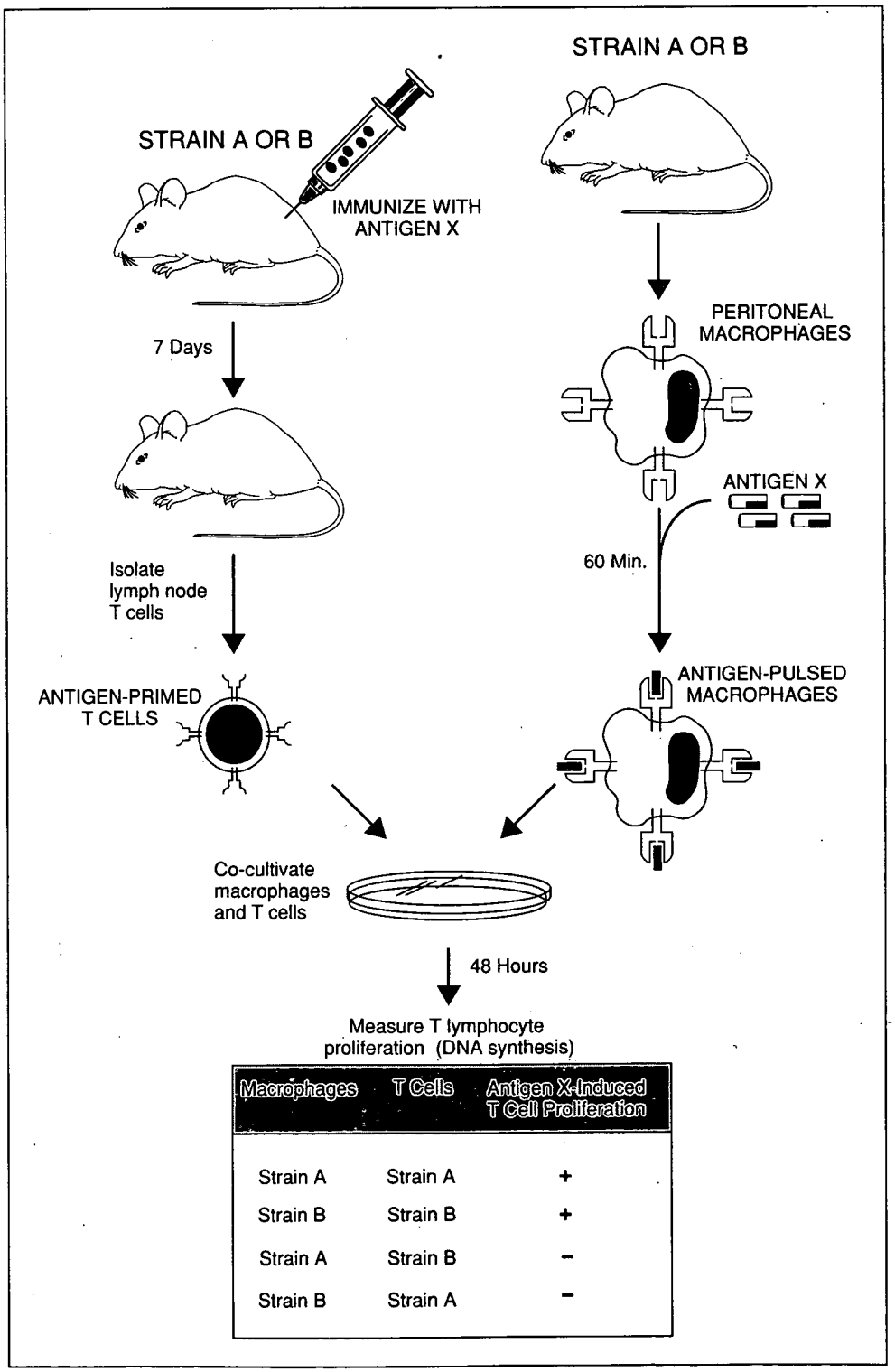
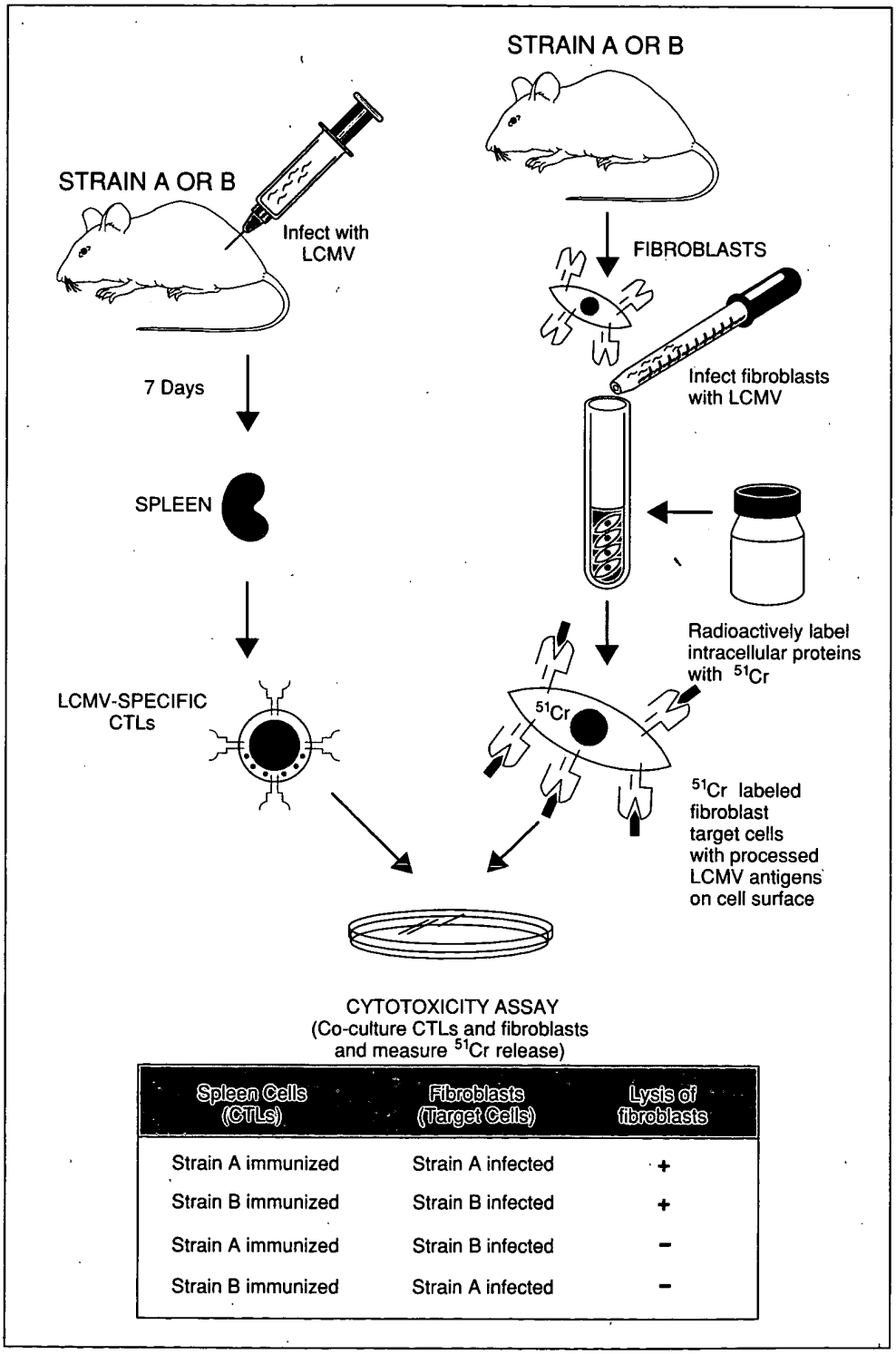


FIGURE 6-1. MHC restriction of proliferating (helper) T lymphocytes. *T cells from a strain A or strain B mouse primed with an antigen X proliferate in response to that antigen only in the presence of strain A or B macrophages (or other antigen-presenting cells [APCs]), respectively. In the experiment depicted, the T cell populations are devoid of alloreactivity; i.e., strain A T cells do not respond to the foreign MHC molecules of strain B, and vice versa. The T cells also do not proliferate in the absence of antigen. Using congenic and recombinant strains of mice, it can be shown that the macrophages and T cells must come from animals that share class II MHC alleles in order for the antigen-induced T cell proliferation to occur.*

adoptive transfer techniques and *in vitro* studies of antibody production showed that helper T lymphocytes and B cells cooperate to produce an antibody response to a protein antigen only if the B cells express class II MHC molecules that are seen as self by the T cells. This phenomenon is discussed in more detail in Chapter 9. It further supports the conclusion that helper T cells are class II MHC-restricted.

3. Perhaps the clearest demonstration of MHC restriction came from assays of virus-specific CTL-mediated lysis of virally infected target cells in mice and humans. In most of these systems, the virus-infected target cells are lysed only if they express class I MHC molecules that are recognized as self MHC by the T cells (Fig. 6-2). This established that *CTL recognition of viral antigens is class I MHC-restricted.*

These experiments suggest that the MHC gene products involved in T cell antigen recognition must



Spleen Cells (CTLs)	Fibroblasts (Target Cells)	Lysis of fibroblasts
Strain A immunized	Strain A infected	+
Strain B immunized	Strain B infected	+
Strain A immunized	Strain B infected	−
Strain B immunized	Strain A infected	−

FIGURE 6-2. MHC restriction of cytolytic T lymphocytes (CTLs). *Virus-specific CTLs from a strain A or strain B mouse lyse only syngeneic target cells infected with the specific virus. The CTLs do not lyse uninfected targets and are not alloreactive. Further analysis has shown that the CTLs and target cells must come from animals that share class I MHC alleles in order for the target cell to present viral antigens to the CTLs. LCMV, lymphocytic choriomeningitis virus.*

exogenously encountered proteins. Second, the adenovirus E19 protein specifically binds to and prevents transport of class I MHC molecules out of the ER. The ability of E19 to block nascent class I transport correlates with its ability to block class I-restricted antigen presentation.

Thus, the association of antigens with class I versus class II MHC molecules is due to the trafficking of the antigens through different intracellular compartments (Fig. 6-11). In most cases, the commitment to one or another traffic pattern is determined by where the antigen comes from; endogenously synthesized antigens end up associated with class I MHC and exogenously synthesized and endocytosed antigens end up associated with class II MHC. There are exceptions, however, when endogenously synthesized proteins do end up being presented in association with class II MHC molecules.

Many unanswered questions remain about the cell biology of class I-restricted antigen presentation. The site at which antigens are processed into peptides before association with class I MHC molecules is not known, nor is it understood how the processed peptides get into the rough ER, where nascent class I MHC molecules are being synthesized. There is some evidence that the ER itself may contain proteolytic enzymes that could generate immunogenic peptides. Since both class I and class II MHC molecules are produced in the rough ER and both have a natural affinity for peptides, there must also be some mechanism that prevents peptides from binding to class II molecules in the ER. This may be accomplished by the class II-associated invariant or γ chains that may interfere with the peptide-binding clefts. Class I MHC molecules do not have invariant chains when they are synthesized and are therefore free to bind peptides produced within the cell. This may be one reason why

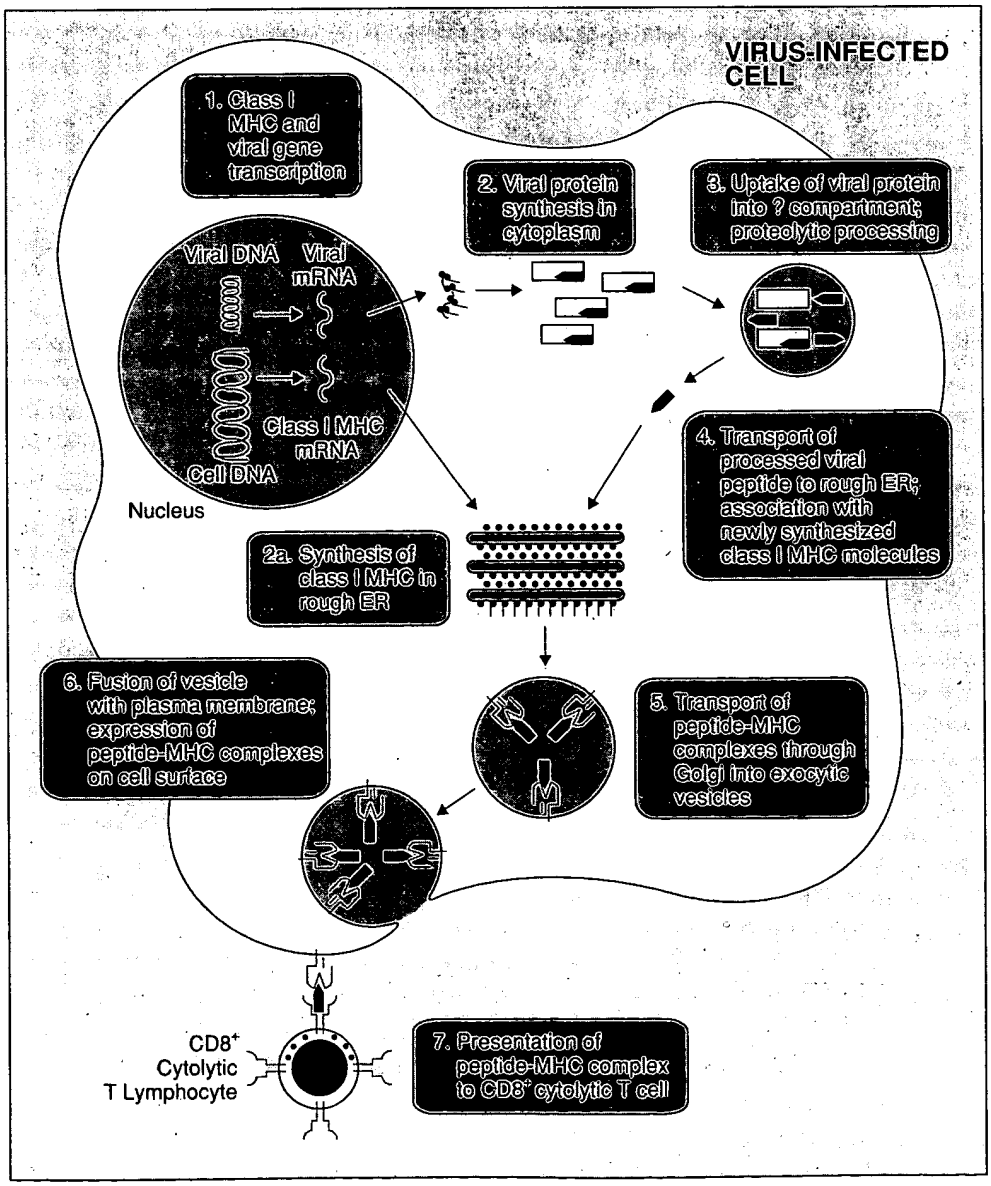


FIGURE 6-11. *Pathway of class I MHC-restricted presentation of an endogenously synthesized (e.g., viral) antigen.*

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